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DEVELOPMENT
OF AN
ULTRA-FAST-CURING WOUND DRESSING

FINAL REPORT

Michael Szycher, Ph.D. and Jonathan L. Rolfe

December 15, 1986

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I. SUMMARY

Thermedics Inc. is developing a second-generation, drug-dispensing wound dressing. The wound dressing, which can be applied by the wounded soldier himself, incorporates thrombin as a coagulant, and gentamycin sulfate as a wide-spectrum antibiotic.

The new wound dressing is a trilaminate composite. The air side of the trilaminate is a fabric impregnated with an aliphatic, medical grade polyurethane elastomer; the middle laminate is a controlled release layer, containing the microencapsulated pharmacoactive agents, and the third laminate is a 1.0-mil-thick layer of acrylic-based, pressure-sensitive adhesive.

The middle layer is fabricated from a mixture of urethane and silicone oligomers, which are precompounded with pharmacoactive agents, and is subsequently solidified (cured) upon mere exposure to low-intensity UV radiation at room temperature. Solidification at room temperature is a vital consideration, because most drugs are rapidly inactivated upon mild heating. Once cured, the oligomer layer containing pharmacoactive agents becomes a controlled-release monolith, capable of dispensing drugs at a continuous and predictable rate.

II. FOREWORD

Future conflicts may have to be fought without the advantage of overwhelming American air supremacy. In the absence of air supremacy, it may not be possible to evacuate wounded American soldiers for proper medical treatment for at least several days. This situation implies that a wounded soldier would need to be treated in the field; the initial treatment would have to be performed by himself, a buddy, or by a paramedic.

Based on this scenario, we embarked on the development of a new field wound dressing. The new field wound dressing would need to be applied without the benefit of prior medical training, during combat, and under all imaginable climatic conditions. Furthermore, the new wound dressing needs to incorporate coagulants and extended-action therapeutic agents to provide immediate stabilization of the wound. Currently available hospital wound dressings do not meet these requirements.

Under research contract DAMD17-83-C-3240, Thermedics is developing a second-generation wound dressing which speeds wound healing, incorporates pharmacoactive substances, and can be easily applied by the wounded soldier himself. This new wound dressing is based on an ultra-fast-curing liquid polyurethane oligomer. The oligomer can be easily precompounded with pharmacoactive agents and, subsequently, cured in less than seconds at room temperature by illumination with UV radiation. Following cure, the wound dressing delivers the pharmacoactive agents in a controlled, sustained-release basis.

This second-generation, medicated wound dressing, when properly developed and tested, may become an ideal vehicle for the initial wound stabilization of wounded soldiers. Our research is being aimed at the development of medicated wound dressing with the following characteristics:

- Oligomer cured at room temperature during manufacture; thus, even heat-sensitive drugs may be incorporated.
- The ready-to-use field wound dressing will be dispensed from waterproof kits carried in a standard-issue backpack.
- Field wound dressing may be applied under any conceivable climatic condition by nonmedical personnel.
- Dressing is highly compliant for physical comfort and is highly abrasion resistant, even when wet.
- Dressing is moisture permeable but does not permit penetration of water or bacteria.
- Dressing delivers medicaments on a controlled, predictable and sustained basis.

This unique combination of properties makes our new field wound dressing an innovative solution to the changing military medical priorities.

III. MILITARY SIGNIFICANCE

Contingency plans for future conflicts place unique demands on the military which are not experienced in the civilian community. Contrary to the present treatment rendered to most casualties, it is most probable that soldiers wounded in future combat environments will face an entirely different situation. It will be common for evacuation of these patients to be delayed for 72 hours and possibly longer. During this critical post-wounding period, qualified medical personnel will not be immediately available to initiate therapy. It is during this crucial time that care will be self-administered or at best be provided by minimally trained personnel. It thus becomes critical that means be available to initiate therapeutic measures under these unusual circumstances.

Less than half the number of soldiers killed in battle die outright as a result of explosions or high-velocity missiles. The high morbidity and mortality associated with combat injuries is primarily attributable to post-wound medical complications, such as overwhelming infections and uncontrolled bleeding. Traditionally, wounds have been treated with dressings. Wound dressings are usually composed of sterile, absorbent cloth, pressure bandages, or a flat strip of elasticized, adhesive film, designed to cover and protect wounds.

The vast majority of maxillofacial wounds inflicted in combat are infected or become infected early on in their course of treatment. Currently available wound dressings are primarily limited to gauze pressure bandages. These materials have minimal beneficial characteristics. They function as simple coverings that are not impervious to microorganisms, thereby providing little protection from infection. By being absorbent, they may tend to desiccate the wound thus delaying healing. The material absorbed into the dressing may provide an excellent substrate supporting microbial growth. These materials may provide a mild measure of hemorrhage control by the application of pressure. However, pressure must be maintained for long periods, thereby restricting body movement so important in combat.

It is our intent to provide a compliant, thin, easily applied medicated wound dressing, dispensed from water-impermeable packages. The medicated wound dressing would be applied to maxillofacial wounds to stop bleeding and prevent bacterial infection, thus providing immediate stabilization of wounds until more definitive medical attention becomes available to the soldier.

Under these circumstances, a wounded soldier can return to combat with the comforting knowledge that the wound dressing is delivering a precise, controlled, and reproducible amount of coagulant and antibiotic. Further, the highly compliant wound dressing will reduce abrasion pain and will not interfere with normal body movements.

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IV. RESEARCH OBJECTIVES

A. IDEAL REQUIREMENTS

The ideal second-generation medicated field wound dressing should:

- Be soft and elastic, closely mimicking the mechanical properties of natural, intact skin.
- Display adequate adhesion to intact skin, but be minimally adhering to clot, so it may be removed at will.
- Control water vapor and oxygen exchange, thus maintaining a moist environment for rapid healing.
- Gradually deliver broad-spectrum antimicrobial agents that are nontoxic to the injured tissue.
- Deliver a bolus of coagulant, to stop and control bleeding for prolonged periods.

These research objectives are aimed at the development of a field wound dressing that provides immediate wound stabilization. This wound stabilization is expected to be accomplished through: (a) reduction of abrasion trauma, (b) easy removal without precipitating another bleeding episode, (c) promotion of normal wound healing under moist, aseptic environment, (d) prevention of bacterial infection, and (e) efficient return to hemostasis by hemorrhage control.

Incorporation of pharmacoactive agents is a key feature of the new wound dressing. The microencapsulation of drugs into a polymeric matrix was made possible by the development of a room-temperature, ultra-fast UV-curable liquid polyurethane oligomer. This is a crucial consideration, since most drugs are rapidly inactivated by mild heat. To insure full pharmacological activity, the drugs should not be subjected to heat. This requirement was met by incorporating the drugs into the liquid matrix of the uncured oligomer followed by a room-temperature, UV-cure of the dressing.

Once cured, the wound dressing, containing drugs, becomes a sustained-release formulation. The dressing, in turn, once in contact with the wound and bodily fluids, provides immediate, direct, and controlled doses of drugs, targeted to the wound site, thus minimizing problems inherent in systemic drug delivery.

Promotion of the normal wound healing mechanism is another feature of the new field wound dressing. The dressing is semi-occlusive; i.e., it allows O_2 , CO_2 and water vapor to permeate in physiological amounts, but it excludes bacteria. This feature is important because, under these conditions, the field dressing is capable of maintaining the wound moist, but aseptic. And, as explained in the following paragraphs, it is now apparent that moist, aseptic wounds heal faster.

V. HYPOTHESES

Our research objectives are based on the hypothesis that an elastic, semioclusive wound dressing, containing extended-action pharmacological agents will provide soldiers immediate wound stabilization. Our assumptions are that immediate wound stabilization will be accomplished through: (a) hemostasis, (b) control of infection, and (c) promotion of normal wound healing mechanisms.

We hypothesize that hemostasis will be rapidly reached through the incorporation of a coagulant such as thrombostat (lyophilized thrombin). Infection control (from pathogenic bacteria, opportunistic invaders), will be accomplished by incorporation of pharmacological agent(s), such as gentamycin sulfate. Finally, promotion of normal wound healing mechanisms will be accomplished by the use of an abrasion-resistant, field-curable, polymeric membrane, which is: (a) noninflammatory and non-antigenic to the wound, (b) as compliant as skin, (c) similar to skin in oxygen permeability, and (d) similar to skin in water vapor transmission characteristics, thus maintaining an aseptic, moist environment.

For centuries, the common understanding of wound healing remained relatively static. There was an awareness that an open wound was subject to the threat of infection. Optimal wound healing was thought to occur under a scab. Dressings were used as protection from bacterial invasion and infection. Dressing materials, traditionally composed of gauze, encouraged the drying of wounds to facilitate scab formation.

In the 1950s, observers realized that an unbroken blister healed more rapidly. Since the blister protects the wound surface with a layer of fluid, this realization led to a new understanding of wound healing.

Healing of partial-thickness damage has three major steps:

1. Epithelial Proliferation
2. Epithelial Migration
3. Dermal Proliferation

Complete epithelialization (steps 1 and 2) represents an effectively closed wound. The epidermal migration necessary to accomplish this closure is now understood to occur only over moist and healthy tissue.

Research in the 1960s, and published articles of the early 1970s, showed that the optimum conditions for steps 1 and 2 above (epithelialization) occurred under a dressing that maintained a moist environment. The development of the polyurethane products (a temporary artificial skin) arose from the recognition of this wound healing principle. The materials were utilized in the attempt to provide a moist environment much like nature's blister.

Prior to the studies on the potential effects of dressings on the repair process mentioned above, the medical community had thought that the surgical dressing mainly absorbed exudate, cushioned the wound site, and hid the site from the patient. That research illustrated that dressings can affect the response to the wound and even retard healing through dehydration or tissue damage during removal. It is now appreciated that dressings can serve to promote faster healing. They can optimize epithelialization, reduce pain (which is associated with wound dehydration), and minimize local inflammation. If impregnated with drugs, they can also deliver medication at a controlled rate.

Optimal wound healing occurs when the dressing material strikes a balance between dehydration and maceration (which results from accumulation of excess exudate). In addition to stimulating pain, dehydration leads to desiccation and cell death, undermining epithelial movement and wound closure. Prevention of dehydration can minimize eschar formation and inflammatory response. Maceration, which is stimulated by excess fluids and debris, is often accompanied by bacterial proliferation; it also has its own attendant negative effects on wound healing.

We have carefully studied the desired balance between dehydration and maceration. We have thus selected an optimal balance between the moist healing environment (to counter dehydration), and vapor permeability (to counter maceration). A key element in our wound dressing development has been our selection of the most appropriate combination of vapor permeable polyurethane and acrylic, pressure-sensitive adhesive to produce a "second generation" wound dressing.

Therefore, another of our research goals is currently directed toward producing a "second generation" wound dressing capable of producing an aseptic microenvironment under the wound which is most conducive to rapid healing.

VI. WORK TO DATE

Our wound dressing is a trilaminate composite, shown in Figure VI-1. The air side of the trilaminate is a polyurethane-impregnated fabric. The middle laminate is a controlled-release layer containing the micro-encapsulated pharmacoactive agents; and the third laminate is a 1.0-mil-thick layer of acrylic-based, pressure-sensitive adhesive. The entire trilaminate composite is attached to release paper; prior to use, the soldier removes the release paper, and the wound dressing is applied to the wound. The dressing is held onto intact skin by means of the pressure-sensitive adhesive.

The fabric was specifically selected for its ability to stretch like skin. Intact, healthy skin is anisotropic; that is, it stretches more in one direction than in another. The fabric mimics this property and, as a result, the new wound dressing is very comfortable once applied, because it "gives" like skin. In addition, incorporation of the fabric into the dressing imparts drapability previously unattainable by commercially available thin-film wound dressings, since it does not wrinkle when the bandage is removed from the release paper.

However, the most important technical breakthrough of the new wound dressing is our development of non-toxic, tissue-compatible oligomers which cure under UV radiation. Curing by UV radiation is a breakthrough in medical-grade polymer technology, since it allows ultra-fast curing (solidification) of biocompatible materials in a matter of seconds at room temperature.

Utilization of UV-curing oligomers permits the production of ingenious drug-dispensing wound dressings. The liquid oligomer may be compounded with pharmacoactive agents, yet it will solidify upon mere exposure to low-intensity UV radiation at room temperature. Solidification at room temperature is a vital consideration, because most drugs are rapidly inactivated upon mild heating. Once cured, the oligomer containing pharmacoactive agents becomes a controlled-release monolith, capable of dispensing drugs at a continuous and predictable rate.

In our technology, we utilize a mixture of two oligomers: (1) a vinyl-terminated polyurethane, and (2) a vinyl-terminated silicone. The synthesis and compounding of these unique materials are fully described in the paragraphs that follow.

A. SYNTHESIS OF VINYL-TERMINATED POLYURETHANE OLIGOMERS

The polyurethane oligomer comprises a diisocyanate, a macroglycol, and an acrylyl chain terminator which provides the necessary vinyl end groups. Incorporation of a photoinitiator into this oligomer, and subsequent UV bombardment, results in a flexible, elastomeric and highly

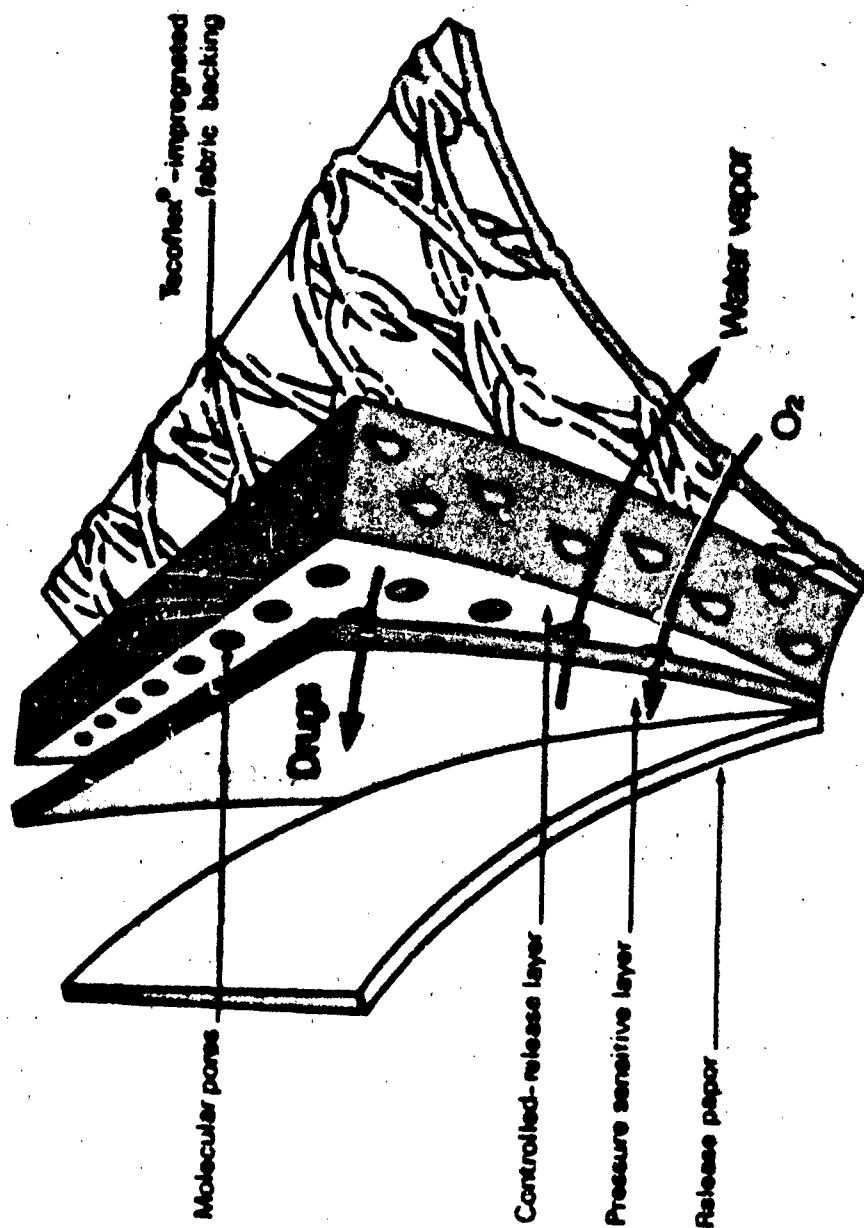


Figure VI.1 The Thermedics wound dressing, containing thrombin and gentamycin sulfate in the middle, controlled-release area

abrasion-resistant cured film. This film is expected to result in a superior field wound dressing, or a "super Band-Aid," when extended-action therapeutic agents are compounded into the oligomer.

Specifically, we have produced UV-curable polyurethane films, with high mechanical properties, such as 1500 psi ultimate tensile strength, 300 percent ultimate elongation, and excellent abrasion resistance by reacting 25.4 percent by weight of isophorone diisocyanate with 57.2 percent of 1000 Dalton polypropylene glycol (PPG). This isocyanate-terminated prepolymer is chain extended with 13.3 percent by weight of hydroxyethyl acrylate. The final product, designated as an oligomer (shown in Figure VI-2) was then further compounded with 3.8 percent by weight of diacetoxyacetobenzophenone (the photoinitiator). The photoinitiator is activated under UV illumination to produce two free radicals, as shown in Figure VI-3. Polymer curing proceeds at room temperature when the free radicals generated by the photoinitiator react with the vinyl end groups, resulting in additional polymerization.

The above constituents were emulsified and cast onto 250- μ m-thick films and exposed for 60 seconds to UV radiation from a commercially available UV source to produce the above-mentioned mechanical properties.

During the first year, we synthesized a variety of urethane oligomers to maximize those properties most desirable in a field wound dressing, such as tensile strength and hardness. In our trials, we need only vary the molecular weight of the PPG to reduce the experimental matrix.

In varying the molecular weight of the PPG, we were guided by well-known principles in polymer chemistry. These principles state that as the molecular weight of the PPG decreases, the tensile strength and hardness decrease concomitantly. Inversely, as the molecular weight of the PPG increases, the tensile strength and hardness increase, thus allowing us to tailor the properties of the finished, cured film.

B. SYNTHESIS OF VINYL-TERMINATED SILICONE OLIGOMERS

The second step in our development program was the synthesis of vinyl-terminated silicone oligomers. These high-molecular-weight silicone oligomers are important in the development of a suitable field wound dressing for two important reasons. First, the higher the silicon content, the lower the adhesion of the dressing to the wound. Second, silicone oligomers can be synthesized in very high molecular weight (high viscosities), thereby providing convenient thixotropic properties to the uncured dressing.

The synthesis of vinyl-terminated silicone elastomers (specifically vinyl-capped alkyl siloxane copolymers) was undertaken in our laboratories according to a proprietary series of steps. Our approach consisted

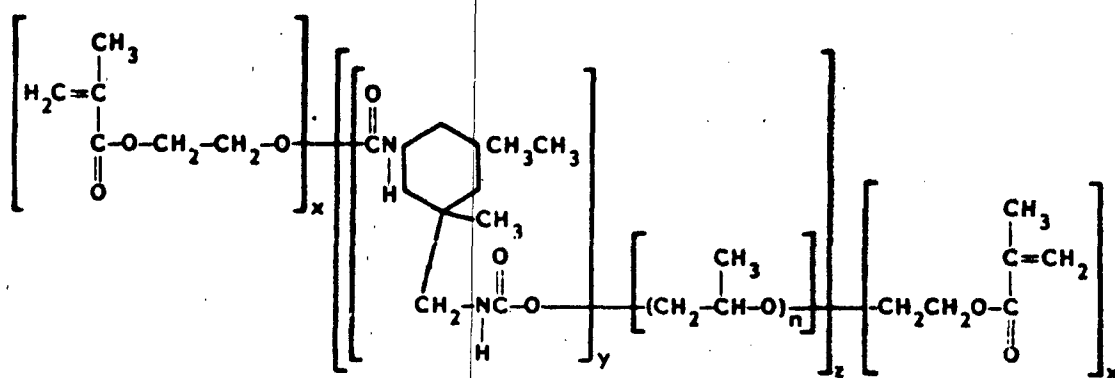


Figure VI-2. Acrylic-Terminated Polyurethane Oligomer

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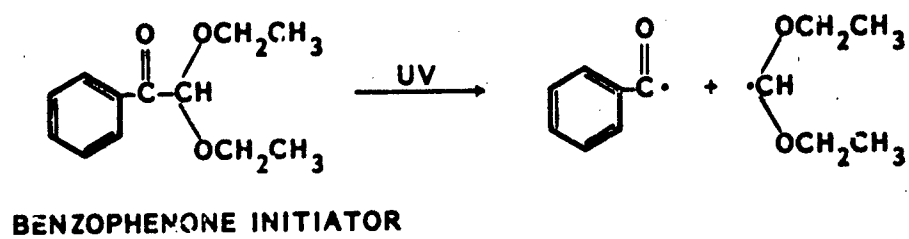


Figure VI-3. UV-Induced Free Radical Formation

of copolymerizing stoichiometric mixtures of octamethylcyclotetrasiloxane and tetramethylvinylcyclotetrasiloxane. The initial polymerization takes place under the influence of a catalyst (KOH), which is subsequently neutralized by the formation of potassium acetate (KAC). Following the removal of H_2O by a vacuum process, the silicone fluid is copolymerized with tetramethylvinylcyclotetrasiloxane to form a vinyl-terminated silicone oligomer.

The copolymerization begins with the anionic, ring opening polymerization of octamethylcyclotetrasiloxane (D_4), the cyclic tetramer of Polydimethylsiloxane (PDMS). This reaction is shown in Figure VI-4.

The initial product of the ring opening polymerization is an equilibrium mixture of cyclic and linear PDMS, with a mean molecular weight determined by the amount of alkali metal catalyst employed. Neutralization, repeated washings, and thorough vacuum stripping at elevated temperatures yield a pure silanol-terminated PDMS fluid, with fluid viscosities increasing with increasing M . For instance, at $n = M_w = 3600$, methyl-terminated PDMS has a viscosity of 60 centistokes; at $n = 1400$ ($M_w = 10,000$) the kinematic viscosity is 10,000 centistokes.

The PDMS was next made to copolymerize with tetramethylvinylcyclotetrasiloxane to produce a high-viscosity, vinyl-terminated silicone oligomer ($> 6,000,000$ cP). The overall initial formula for the synthesis of a silicone oligomer proceeds as follows:

Octamethylcyclotetrasiloxane	100 moles
Tetramethylvinylcyclotetrasiloxane	0.3 moles
Analytical-Grade Potassium Hydroxide (Reacted at 145°C for 5 hours)	0.001% by weight

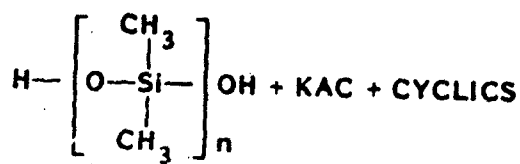
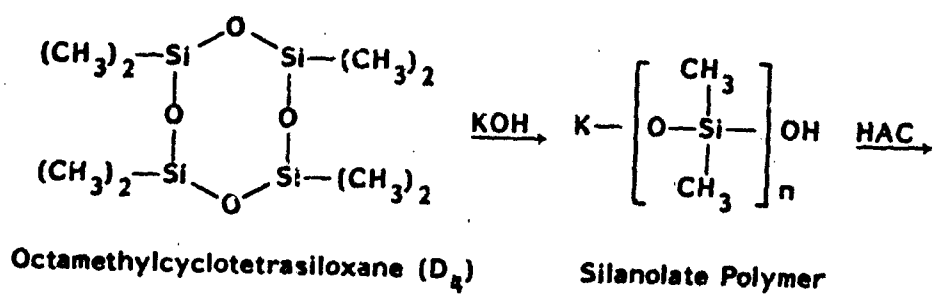
The vinyl-terminated silicone oligomer can be cured into a soft, pliable elastomer by exposure to UV bombardment via free radical addition polymerization, according to the reaction shown in Figure VI-5.

Using this procedure, we have successfully UV cured vinyl-terminated silicone oligomers, which have proven to be biocompatible in preliminary animal experiments. Typical physical properties of the fully cured silicone elastomers are summarized in Table VI-1.

C. COMPOUNDING

The urethane and silicone oligomers are intimately mixed in a heated two-roll mill. The gentle shearing action of the rotating rolls results in a thorough dispersion of the two liquids.

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Silanol-Terminated Polymer, Raw

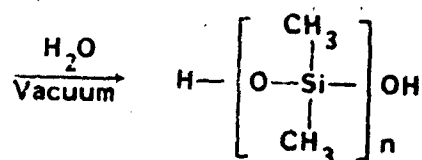


Figure VI-4. Pure, Silanol-Terminated Di Methyl Siloxane Silicone Fluid Via Ring Opening of Octamethylcyclotetrasiloxane

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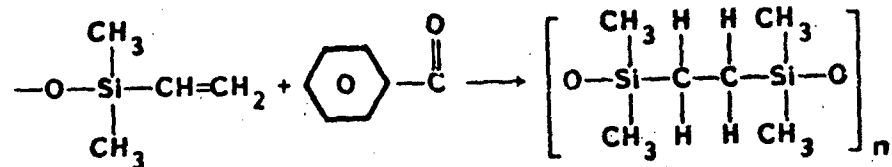


Figure VI-5. Free Radical Polymerization of a Vinyl-Terminated Silicone Gum

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TABLE VI-1
TYPICAL PROPERTIES OF AN
ELASTOMERIC SILICONE POLYMER

Hardness (Shore A)	60
Tensile Strength (psi)	1000
Elongation (%)	300
Tear Strength (pli)	175

Once adequate dispersion of the two oligomers has been achieved, thrombin and gentamycin sulfate are slowly added to the rolling bank. The mill is then used to disperse the pharmacoeactive agents in a highly controlled fashion; mixing, dispersion and microencapsulation of the drug powder are attained in approximately 20 minutes. After the 20 minutes have elapsed, a microencapsulated powder (average particle size = 0.71 μ m) is obtained.

At this time all fluorescent lights (which emit weak UV radiation) are shut off. In a darkened laboratory, with only lights from red bulbs (similar to those used in photographic darkrooms), the photoinitiator is added to the oligomer/drug mixture. An additional period of 10 minutes of mixing in the mill is required for complete solution. At the end of this operation, the uncured oligomer/drug/photoinitiator mixture is a thixotropic mixture, ready for curing.

The mixture is next applied in the form of a 250- μ m-thick membrane onto the TECOFLEX[®]-saturated fabric. The oligomer layer is cured by illumination from UV-curing lamps, emitting a radiation spectrum covering the range from 320 to 440 nm. The curing is performed in continuous ovens, in a nitrogen atmosphere to protect the uncured membrane from oxygen, moisture, or other airborne contaminants.

D. PRESSURE-SENSITIVE ADHESIVE

To apply the wound dressing, and to keep it in place for the desired time period, a pressure-sensitive adhesive must be used. Delivery of pharmacoeactive agents by a medicated wound dressing utilizing a pressure-sensitive adhesive to maintain effective wound contact demands the marriage of three unrelated disciplines: pharmaceutical technology, polymer technology, and pressure-sensitive-adhesive technology.

In our development of the field wound dressing, we have been guided by a number of key principles. These principles are encompassed in pharmaceutical, polymer, and adhesive technologies, and will be discussed in the following order: (1) adhesion to skin, (2) cohesive strength, (3) anchorage of adhesives, (4) skin irritation, (5) drug-oligomer-adhesive interaction, and (6) shelf stability.

1. Adhesion to Skin

Skin adhesion is a fundamental property required to hold any device in place. However, adhesive properties should be such that the dressing can be removed after the required residence time in an unremarkable manner. Skin adhesion should be balanced between: (a) the adhesion level required for secure holding regardless of patient movement, perspiration level or bathing, and (b) ease of removal when dosage is complete.

The most desirable adhesive system will also show uniform adhesion to skin vs time with only moderate adhesion buildup or loss. Also, the range of values observed should be statistically reproducible and as small as possible.

Adhesion level to the patient dosage site with a wound dressing should only be enough to effectively keep the device in place for the necessary dosage period. Higher levels of skin adhesion should be avoided when possible, since high skin adhesion levels increase the incidence of excoriation during removal. Higher than necessary levels of skin adhesion also increased the probability of skin sensitization and irritation with repeated use on the same site.

In our medicated wound dressing, we have chosen an acrylic-based, pressure-sensitive adhesive that builds adhesive to the skin site rapidly, plateaus, and thereafter maintains uniform adhesion for up to seven days. Upon removal, we have observed a minimum of adhesive residue left on the skin site, and removal has been unremarkable.

2. Cohesive Strength

This is the ability of the adhesive to stay together and to stay in place under load, i.e., resist shear. Good cohesive strength is also vital for clean removal from the skin with minimum residue. It is a manifestation of the visco-elastic properties of a particular system.

Cohesive strength or lack thereof is a function of the molecular weight and molecular weight distribution. Addition of relatively low molecular weight tackifying agents to compounded adhesives affects the molecular weight distribution. Adhesive processing during coating can also directly influence final molecular weight distribution.

Positive tests for good cohesive strength in vivo are the unit staying in place on the patient (not sliding) and unremarkable removal with no visible adhesive residue left on the skin.

In our wound dressing, we selected a pressure-sensitive adhesive which displayed sufficient cohesive (or internal) strength to remain in place, yet it peeled from the skin cleanly. Cohesive strength was not adversely affected by either ethylene oxide or gamma radiation sterilization, and was unaffected by temperatures between +95°F and -20°F.

3. Anchorage of Adhesive

The pressure-sensitive adhesive, which is designed to hold a medicated wound dressing to a soldier's skin, must on the obverse side stay adhered to the dressing. Keeping the adhesive firmly attached to the dressing is referred to as adhesive mass anchorage.

Adhesive mass anchorage is most easily tested in a direct manner. The tests are essentially qualitative - you either have it or you don't. An effective test that can be done without instrumentation is to simply fold the adhesive film composite pressure-sensitive side upon itself and press together to insure good contact. Then peel one end back on itself creating a 180 degree peel test.

Acceptable mass anchorage is also demonstrated by lack of adhesive transfer from one surface to the other. Still another sign of good mass anchorage is a uniform adhesive appearance after adhesive separation by the above tests. Unacceptable mass anchorage is gross transfer or delamination of adhesive from its support film or layer.

Mass adhesive anchorage is critical to device performance. Firstly, loss of mass anchorage may cause the device to fall off (in the worst case). Secondly, poor mass anchorage with attendant separation of device layers can cause dosage interruption, and/or dose dumping. In our medicated wound dressing, all components (saturated fabric, oligomer layer and pressure-sensitive adhesive) were carefully chosen for compatibility with the foreseen field service demands.

4. Skin Irritation

It is an unnatural condition for the skin to be covered with an adherent wound dressing, and to keep potential skin irritation, we have considered the following parameters:

- Skin Irritation Potential (Rubber, Silicone, or Acrylic)
- Length of Time Worn
- Drug Adhesive Interaction
- Adhesive Permeability/Porosity

In terms of skin irritation potential, rubber-band adhesives have shown the greatest potential; silicones were excellent, but changed tackiness after gamma sterilization; acrylics offered the best combination of properties.

Acrylics were shown to remain stable in contact with human skin for about seven days, and no drug/adhesive interaction was observed. Acrylics were also the most permeable/porous adhesives tested; this is important, since the more permeable/porous an adhesive is, the more it allows skin to breathe or respire resulting in less tendency toward skin irritation.

This is particularly important in a field wound dressing that may not be replaced for several days. Under these conditions, large patches

of skin will be continually covered by an adhesive dressing, which may lead to skin maceration. The selected acrylic adhesive has demonstrated the lowest tendency toward skin maceration, since it is highly porous.

5. Drug/Adhesive Interactions

The possibility of drug/adhesive interaction is an important consideration as it may change:

- Drug Potency as a Function of Time
- Device Wear Characteristics
- Skin Adhesion
- Skin Irritation

Drug/adhesive interaction can affect skin adhesion. This can manifest itself as a softening of the adhesive mass making it too tacky with loss of cohesive strength. It may also cause irritation due to high skin adhesion. Excessively high drug levels can also work in the opposite direction and dry up the mass with resultant loss of tack or quick-stick.

We are cognizant of skin irritation phenomena which can result from unforeseen drug/adhesive interactions. To date, our tests have shown that drugs maintain their integrity and pharmacological activity when incorporated into our medicated wound dressing system.

6. Shelf Stability

The best designed system is of little value if its performance is lacking when it is finally used. Substandard shelf stability may be manifested by incorrect dose delivery or deterioration of pressure sensitive.

The choice of a pressure-sensitive adhesive polymer system will play a major role in shelf stability of the adhesive component of the delivery device. While there are probably exceptions, it has generally been demonstrated that synthetic rubber/natural rubber resin adhesives deteriorate most quickly with time. Acrylic polymer pressure sensitives show exceptional aging properties, and have thus become our choice for use in the manufacture of medicated field wound dressing.

E. IN VITRO BIOCOMPATIBILITY TESTS

1. Growth Inhibition

African green monkey kidney cells (vero) were grown in Dulbecco's modified eagles medium supplemented with 10 percent fetal bovine serum,

100 mm L-glutamine, 1 percent NCTC 109, 1 percent sodium pyruvate and penn-strep fungizone (DMEM) (M.A. Bioproducts, Walherville, Maryland). Cells were harvested by trypsin versine treatment, washed three times with Hank's balanced salt solution (HBSS) and viability count determined by trypan blue exclusion.

The vero cells were next suspended in DMEM at a concentration of 1×10^6 viable cells per ml. At this time 300 ml of the cell suspension was placed in each well of 24 well tissue culture plates; the plates were next incubated for 3 hours at 37°C in 10 percent CO₂ and 100 percent humidity to allow cell adhesion. The media and non-adherent cells were then removed by aspiration and 300 ml of fresh DMEM was added to each well.

Samples of wound dressing were cut into 4 mm squares and placed in direct contact with the bottom of each well. The following samples were evaluated in triplicate: precured wound dressings containing either 2, 4 or 6 percent photoinitiator; uncured wound dressings containing either 2, 4 or 6 percent photoinitiator; and uncured wound dressings which were then cured in situ by exposure to UV light. All samples were evaluated visually at 24, 48, 72 and 96 hours for estimation of cell growth in approximation to the wound dressing by phase contrast microscopy. In addition, 100 ml of the media was removed from each well at each time frame, 100 ml of fresh media was added as a replacement, and the media mixed with trypan blue. All samples were placed in a hemocytometer and the number of non-viable cells/100 ml were determined and compared to control wells which had been treated in an identical manner with the exception that no wound dressings had been placed in the wells.

At all observation periods, all formulations of the wound dressings appeared similar. It was observed that cell density increased as incubation time was extended. By 96 hours, all samples displayed cell monolayers that appeared to be in intimate contact with the wound dressing margins.

The number of non-viable cells observed in media samples which had been exposed to the wound dressings was similar to the control wells (Table VI-2). As would be expected, the number of non-viable cells/100 ml of media increased in both experimental and control wells as the incubation time was extended. These data suggest that the wound dressings tested had minimal to no adverse effect of tissue culture cells.

2. Cytotoxicity (Chromium Release Method)

Vero cells were cultured and harvested as before, suspended in DMEM and total viable count was determined by trypan blue exclusion.

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TABLE VI-2
NON-VIABLE CELLS/100 ml OF
MEDIA REMOVED FROM TISSUE CULTURE WELLS

	Incubation Time (hr)			
	24	48	72	96
Control wells N=12	0.16*	0.47	0.93	1.82
Experimental wells N=24	0.21	0.46	1.01	1.79

*Mean non-viable cells per 100 ml.

All samples used in this procedure displayed >98 percent viability. Following centrifugation, the cells were suspended at a concentration of 1×10^6 cells/ml and were labelled with sodium chromate (^{51}Cr) with approximately $1.5 \mu\text{C}$ per 10^6 cells. The sample was incubated for 90 minutes at 37°C in 10 percent CO_2 , washed two times in HBSS and resuspended in DMEM. Following a 1-hour incubation, cells were again washed two times in HBSS and aliquated in DMEM at either 60,000, 30,000 or 15,000 cells/200 ml. Samples (200 ml) of each cell concentration were plated in each well of 96-well tissue culture plates and exposed to either 1 percent SDS to determine total ^{51}Cr release, no exposure to determine spontaneous release and to 2 mm^2 segments of selected wound dressings as the experimental group. Following a 24-hour incubation, the culture supernatant was removed using the TiterTech Supernatant Collection System and ^{51}Cr release was determined for all samples using a Model 1185 Gamma Comm (Tracor Analytic, Elhr Grove Village, Illinois).

The data obtained (Table VI-3) for all samples were similar. As the cell concentration decreased, the counts per minute (CPM) decreased proportionally. When the CPM of the experimental group was compared to the CPM in the spontaneous release group, the data were indistinguishable. Both groups displayed approximately 20 percent of the CPM obtained in the total release groups.

Within the limits of these studies it would appear that the previously mentioned wound dressings are non-cytotoxic to tissue culture cells in vitro thus suggesting that one would expect no cytotoxicity in vivo. It would appear that these wound dressings would be biocompatible in an in vivo model system.

F. IN VIVO PERFORMANCE TESTS

In accordance with the provisions of Contract No. DAMD-17-83-C-3240, we have been supplying prototype samples of medicated field wound dressings to Dr. J. Vincent at USAIDR for in vivo evaluation. These prototype dressings contained either 8 mg or 16 mg of gentamicin sulfate with a Poly Ethylene Glycol (PEG) excipient, and were supplied as pre-sterilized, one-inch-square, unsupported, free films.

Results to date have been very encouraging, and are fully described below. Basically, Dr. Vincent reported a classical dose-response curve, with control (placebo) animals displaying a mean count of 2.1×10^7 cfu/in² (colony forming units per square inch) of Staphylococcus aureus (ATCC 12600) after 3 days; animals treated with dressings containing 8 mg of microencapsulated gentamicin sulfate had a mean count of 6.99×10^4 cfu/in²; and, finally, animals treated with dressings containing 16 mg of microencapsulated gentamicin sulfate had a mean count of only 9.81×10^2 cfu/in².

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TABLE VI-3
⁵¹Cr RELEASE EXPRESSED AS MEAN CPM FOLLOWING
24 HOUR INCUBATION

Cell Concentration	Total Release	Spontaneous Release	Experimental Release
60,000/well	56,783*	10,179*	9569*
30,000/well	29,172	5,247	4598
15,000/well	16,427	2,506	2470

*Mean CPM. N=3 for each group tested.

General impressions to date are that wound dressings made under this program were very favorable, and conformed well to the wounds. The dressings were slightly friable which was expected, since they were fabricated without the TECOFLEX TS backing material to facilitate extraction of residual gentamicin. So far, the only problem detected in these studies was a tendency of the prototype dressings to adhere to the wounds causing disruption upon removal; this problem will be addressed in the next contract year.

According to the protocol selected at USAIDR, guinea pigs are anesthetized and the intrascapular area shaved and treated with a depilatory. After injection of 0.5 ml of mepivacaine HCl at the operative site, a full-thickness dissection, approximately one inch square, is performed. The surgical phase is followed by the inoculation of Staphylococcus aureus at a concentration of 3×10^{11} per ml. A total inoculation of 50 μ l is given, resulting in a 1.5×10^9 cfu/in². All wounds are covered with polyethylene sheeting, and the animals are checked daily for three days intraeroscopically for induration, erythema, moisture, supuration and dressing removal.

At the end of the third day, the quantitative sampling technique of Williamson and Klingman is used to determine accurately the wound microflora. This is accomplished by holding a 2-cm² sterile glass chamber to the wound surface, and scrubbing the site for one minute using a sterile Teflon policeman and a 1-ml solution of buffered Triton X-100. The wash fluid is aspirated, replaced with 1 ml of fresh scrub solution, and the process repeated. The animals are then euthanatized with an overdose of Nembutal.

A 0.1 ml aliquot of the scrub solution is serially diluted in tenfold increments and plated on Trypticase Soy Agar by the Spiral Plater System. Following incubation of plates, the bacteria are counted with a Laser Bacteria Colony Counter, and the number of microorganisms/in² are calculated. Results are reported as colony forming units per square inch.

Following the above protocol, the Thermedics' prototype wound dressings consisted of four groups as shown in Figure VI-6.

A bacterial count of 10^2 may well be optimal for a field wound dressing. Clinical evidence suggests that some bacteria in a three-day wound prevents or retards fungal infection; whereas complete elimination of bacteria may allow opportunistic infection.

The above data were compiled from one of the last experiments conducted at USAIDR on October 25-28, 1985. After the trials were concluded, all loaded wound dressings were placed individually in autoclave bags and heat sealed in preparation for transport to Thermedics

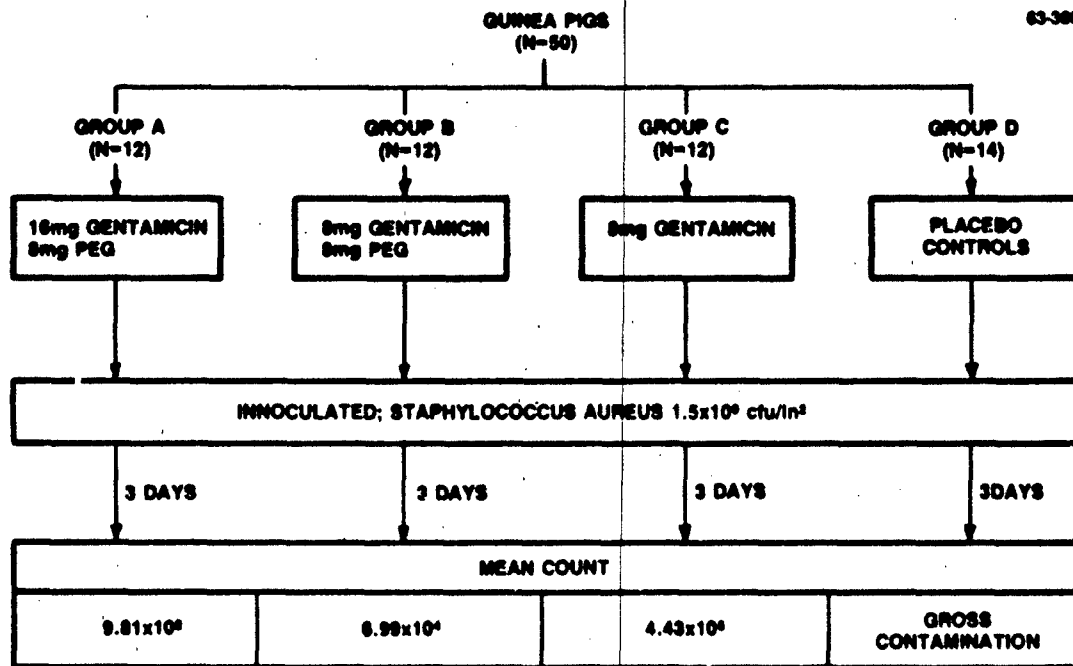


Figure VI-6. Experimental In Vivo Protocol

for extraction and quantification of residual gentamicin sulfate. All wash solutions were serially diluted and plated with the spiral plates for bacterial count. In addition, after clotting, serum was harvested from blood samples for quantification of serum gentamicin. Results are given in Tables VI-4 through VI-7.

These excellent results encourage us to continue the development of gentamicin sulfate medicated field wound dressings. Gentamicin is a member of the aminoglycoside antibiotic group, which also includes streptomycin, neomycin, kanamycin, amikacin, tobramycin and netilmicin. These agents are primarily used for treatment of serious infections caused by gram-negative bacteria in which less toxic antibacterials are ineffective or contraindicated.

Gentamicin was isolated from the actinomycete Micromonospora purpurea and found to have a wide spectrum antibacterial effect. In vitro tests have demonstrated gentamicin to be a bacterial antibiotic active against Escherichia coli, Proteus species, Pseudomonas aeruginosa, species of the Klebsiella-Enterobacter-Serratia group, Citrobacter species and Staphylococcus species (including penicillin- and methicillin-resistant strains). Resistant to gentamicin and the other aminoglycosides are most species of streptococci, particularly group D or viridans, and anaerobic organisms such as Bacteroides and Clostridium.

Gentamicin is found naturally in three chemical forms known as gentamicin C, C_{1a} and C₂, and collectively referred to as gentamicin C, or by its trade name gentamicin. All elicit similar biologic activity, and their aminoglycosidic structures are depicted in Figure VI-7.

Although it is an excellent antibiotic, the greatest single drawback to the wide use of gentamicin is its narrow therapeutic range. Serious infections require peak blood levels of 5 to 8 µg/ml, but levels of 10 to 12 µg/ml must be avoided because of an increased risk of ototoxicity and nephrotoxicity. All studies agree that there is a very narrow range of blood levels between effective and toxic concentrations.

We are fully aware of the potential ototoxicity and nephrotoxicity issues associated with systemic gentamicin therapy. However, in our case we are applying gentamicin on a local basis. Because the gentamicin is released in a controlled, timed-release basis (with no potential for dumping), we are able to maintain a therapeutic antibiotic dose for a sustained time on the wound surface, while never producing a toxic serum level. As our experiments indicate, serum levels of gentamicin in treated animals are undetectable; thus, neither ototoxicity nor nephrotoxicity are expected, even when treating the most extensive wounds.

TABLE VI-4
NUMBER OF STAPHYLOCOCCUS AUREUS cfu/in²,
REMAINING ON WOUND SURFACES
FOLLOWING TREATMENT WITH MEDICATED DRESSING
Group A (16 mg Gentamicin Sulfate; 16 mg PEG)

Animal No.	Wound Dressing Code Number	cfu/in ²	Serum Gentamicin
1	1464	3.51×10^3	0
2	1443	5.17×10^3	0
3	1592	2.07×10^3	0
4	1448	0	0
5	14235	0	0
6	1456	0	0
7	1384	0	0
8	1372	0	0
9	1320	1.03×10^3	0
10	1354	0	0
11	1283	0	0
12	1287	0	0

Mean Count = 9.81×10^2

TABLE VI-5
NUMBER OF STAPHYLOCOCCUS AUREUS cfu/in²,
REMAINING ON WOUND SURFACES
FOLLOWING TREATMENT WITH MEDICATED DRESSING
Group B (8 mg Gentamicin Sulfate; 8 mg PEG)

Animal No.	Wound Dressing Code Number	cfu/in ²	Serum Gentamicin
13	1473	5.58×10^3	0
14	1487	2.21×10^5	0
15	1470	7.73×10^4	0
16	1474	2.48×10^3	0
17	1498	5.38×10^3	0
18	1483	4.14×10^3	0
19	1539	1.49×10^4	0
20	1534	1.77×10^4	0
21	1544	1.06×10^5	0
22	1566	1.83×10^5	0
23	1528	1.84×10^5	0
24	1369	1.85×10^4	0

Mean Count = 6.99×10^4

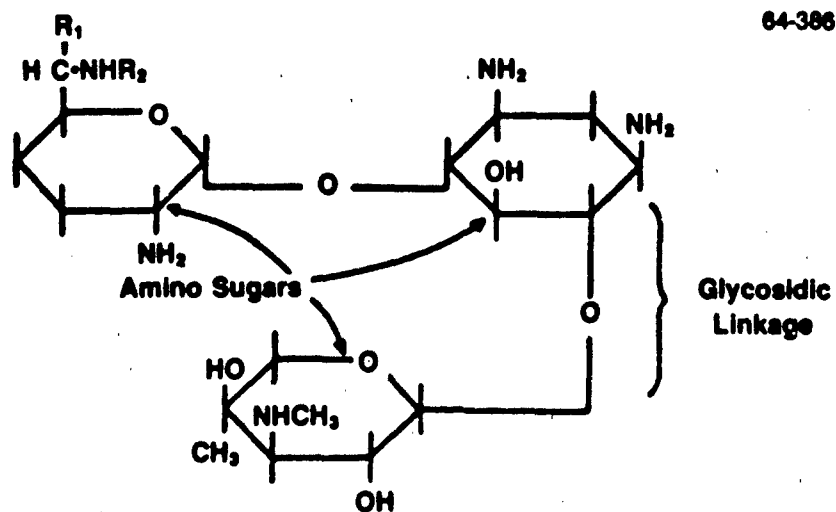
TABLE VI-6
NUMBER OF STAPHYLOCOCCUS AUREUS cfu/in²,
REMAINING ON WOUNDED SURFACES
FOLLOWING TREATMENT WITH MEDICATED DRESSING
Group C (8 mg Gentamicin Sulfate)

Animal No.	Wound Dressing Code Number	cfu/in ²	Serum Gentamicin
25	1656	1.44×10^3	0
26	1659	1.13×10^4	0
27	1696	4.55×10^3	0
28	1652	2.07×10^3	0
29	1570	4.31×10^4	0
30	1583	8.45×10^4	0
31	1434	3.13×10^6	0
32	1454	5.83×10^4	0
33	1276	6.21×10^3	0
34	1279	2.52×10^5	0
35	1289	1.16×10^6	0
36	1284	5.63×10^5	0

TABLE VI-7
 NUMBER OF STAPHYLOCOCCUS AUREUS cfu/in²,
 REMAINING ON WOUNDED SURFACES
 FOLLOWING TREATMENT WITH PLACEBO (CONTROLS)
 Group D (Placebo; Unmedicated Dressings)

Animal No.	Wound Dressing Code Number	cfu/in ²	Serum Gentamicin
37	Polyethylene Sheet	5.63 x 10 ⁶	0
38	Unloaded Dressing	3.40 x 10 ⁷	0
39	Ibid	Biopsy	0
40	Ibid	Biopsy	0
41	Ibid	Biopsy	0
42	Ibid	Gross Contamination	0
43	Ibid	4.66 x 10 ⁷	0
44	Ibid	1.66 x 10 ⁶	0
45	Ibid	Gross Contamination	0
46	Ibid	Gross Contamination	0
47	Ibid	Gross Contamination	0
48	Ibid	Gross Contamination	0
49	Ibid	1.8 x 10 ⁷	0
50	Ibid	Biopsy	0

Mean Count of Six = 2.1 x 10⁷



Gentamicin C

Gentamicin C₁, R₁=R₂=CH₃

Gentamicin C_{1a}, R₁=R₂=H

Gentamicin C₂, R₁=CH₃-, R₂=H

Figure VI-7. Chemical Structure of Gentamicin C
(Gentamicin Powder; Schering-Plough, NJ)

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